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JPW/GJG/YFL
Docket No. 0575/54858

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

September 29, 1999

16675 U.S. PTO
09/29/99

S I R:

Transmitted herewith for filing are the specification and claims of the patent application of:

Howard J. Worman & Naoto Mamiva for
Inventor(s)

HVC CORE PROTEIN BINDING AGENTS FOR TREATMENT OF HEPATITUS C VIRUS INFECTION
Title of Invention

Also enclosed are:

X 12 sheet(s) of informal X formal drawings.

with or declaration of Applicant(s).

power of attorney

assignment of the invention to

X Preliminary Amendment

X verified statement to establish small entity status under 37 C.F.R. § 1.27.

The filing fee is calculated as follows:

CLAIMS AS FILED, LESS ANY CLAIMS CANCELLED BY AMENDMENT

	NUMBER FILED		NUMBER EXTRA*		RATE		FEE	
					SMALL ENTITY	OTHER ENTITY	SMALL ENTITY	OTHER ENTITY
Total Claims	35 -20	=	15	X	\$ 9.00	\$18.00	= \$ 135	\$
Independent Claims	4 -3	=	1	X	\$39.00	\$78.00	= \$ 39	\$
Multiple Dependent Claims Presented: <u>Yes</u> <u>X</u> No					\$130.00	\$260.00	= \$ 0	\$
*If the different in Col. 1 is less than zero, enter "0" in Col. 2					BASIC FEE		\$ 380	\$ 760
					TOTAL FEE		\$ 554	\$

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Applicant or Patentee: Howard J. Worman et al. Attorney's JPW/GJG/YFL
Serial or Patent No.: Not Yet Known Docket No: 0575/54858
Filed or Issued: Herewith
Title of Invention or Patent: HCV CORE PROTEIN BINDING AGENTS FOR TREATMENT OF
HEPATITIS C VIRUS INFECTION

VERIFIED STATEMENT (DECLARATION) CLAIMING
SMALL ENTITY STATUS UNDER 37 C.F.R. §1.9(f)
AND §1.27(d) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

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TYPE OF ORGANIZATION:

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☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
NAME OF STATE: _____
CITATION OF STATUTE: _____

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. §1.9(e)* for purposes of paying reduced fees under 35 U.S.C. §41(a) and 41(b), with regard to the invention entitled

HCV CORE PROTEIN BINDING AGENTS FOR TREATMENT OF HEPATITIS C VIRUS INFECTION

inventor(s) Howard J. Worman et al.

described in:

☒ the specification filed herewith
☐ application serial no. _____ filed _____
☐ patent no. _____ issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive each individual, concern, or organization known to have rights to the invention is listed below^a and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. §1.9(d)* or a nonprofit organization under 37 C.F.R. 1.9(e)*

^a NOTE: Separate verified statements are required from each person, concern, or organization having rights to the invention averring to their status as small entities. 37 C.F.R. §1.27.

Name: NONE
Address: _____

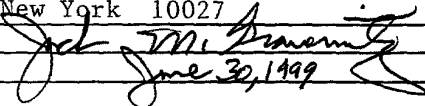
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Applicants: Howard J. Worman and Naoto Mamiya
Serial No.: Not Yet Known
Filed: Herewith

Small Entity/Nonprofit
Page -2-

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. 37 C.F.R. §1.28(b)*.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Jack M. Granowitz
Title In Organization: Executive Director, Columbia Innovation Enterprise
Address: Engineering Terrace - Suite 363, Amsterdam and 120th Street
New York, New York 10027
Signature: 
Date Of Signature: June 30, 1999

*See Reverse

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Howard J. Worman and Naoto Mamiya
U.S. Ser. No. : Not Yet Known
Filing Date : Herewith
For : HCV CORE PROTEIN BINDING AGENTS FOR
TREATMENT OF HEPATITIS C VIRUS INFECTION

1185 Avenue of the Americas
New York, New York 10036
September 29, 1999

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

PRELIMINARY AMENDMENT

Please amend the above-identified application as follows:

In the claims

Please cancel claims 33-44, and 46-69 without prejudice.

No fee is deemed necessary in connection with the filing of this Preliminary Amendment. However, if any fees are required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,



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Application
for
United States Letters Patent

To all whom it may concern:

Be it known that

Howard J. Worman and Naoto Mamiya

have invented certain new and useful improvements in

HCV CORE PROTEIN BINDING AGENTS FOR TREATMENT OF HEPATITIS C
VIRUS INFECTION

of which the following is a full, clear and exact description.

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**HCV CORE PROTEIN BINDING AGENTS FOR
TREATMENT OF HEPATITIS C VIRUS INFECTION**

5 Throughout this application, various publications are
referenced by author and date. Full citations for these
publications may be found listed numerically at the end of
the specification immediately preceding the claims. The
disclosures of these publications in their entirety are
hereby incorporated by reference into this application in
10 order to more fully describe the state of the art as known
to those skilled therein as of the date of the invention
described and claimed herein.

Background of the Invention

15 Chronic hepatitis C is a major public health problem and
one of the leading worldwide causes of chronic liver
disease, cirrhosis and hepatocellular carcinoma (1).
Approximately 4 million Americans are chronically infected
20 with HCV and as many as 25% of them may eventually develop
cirrhosis (2). End-stage liver disease from hepatitis C
is now the leading indication for orthotopic liver
transplantation in the United States. HCV was identified
in 1989 and demonstrated to be the major cause of what was
25 then referred to as non-A, non-B hepatitis (3,4).

The hepatitis C virus (HCV) is a positive single stranded
RNA virus and a member of the *Flaviviridae* family (3,6-
10). Once hepatitis C virus infects cells, the positive,
30 single-stranded RNA genome is translated into a
polyprotein of 3010 to 3033 amino acids, depending upon
the strain (6-9). The viral RNA is not capped and
translation occurs via internal ribosome entry sites
(10,11). The mechanism of translation from uncapped viral
35 RNA therefore differs from that used by virtually all

cellular mRNAs which are capped at their 5' ends.

5 In hepatocytes, the HCV core protein is mostly localized
to endoplasmic reticulum membrane with a large domain
facing the cytoplasm (12). It has been shown to form
multimers (13). The function of HCV core protein in cells
is not clear, however, it may play a role in
transformation and oncogenesis (14). Such a function
could hypothetically arise as a result of interactions
10 with cellular proteins involved in signal transduction or
oncogene or tumor suppressor gene products or by affecting
expression from their genes. HCV core protein may also be
involved in regulating the immune response as it has been
shown to bind to the cytoplasmic domain of lymphotoxin- β
15 receptor (15). Some investigators have also shown that a
truncated portion of HCV core protein can reach the
nucleus (16,17), suggesting that it may directly affect
the expression of cellular genes as demonstrated *in vitro*
(18). It is not clear, however, if this nuclear form is
20 generated in infected cells.

The HCV polyprotein is proteolytically processed by both
host cell and viral proteases into several smaller
polypeptides (6-9,12) (Figure 1). The major structural
25 proteins are a core protein and two envelope proteins (E1
and E2). Four major non-structural proteins called NS2,
NS3, NS4, and NS5, are also generated, two of which, NS4
and NS5, are further processed into smaller polypeptides
called NS4A, NS4B, NS5A, and NS5B. The non-structural
30 proteins have various enzymatic activities, such as RNA
helicase (NS3), protease (NS2, NS3-NS4A complex) and RNA
polymerase (NS5B). NS5A has been implicated in
determining sensitivity to interferon.

35 After cells are infected with a virus, viral proteins can

interact with host cell proteins and influence cell physiology. In previous studies, HCV core protein has been shown to bind to lymphotoxin- β receptor and other tumor necrosis factor receptor family members (15,27). A truncated form of HCV core protein also interacts with ribonucleoprotein K in the nucleus (28). We now show that HCV core protein binds to a cellular RNA helicase and, in experimental systems, inhibits capped RNA translation. This provides a novel mechanism by which HCV may inhibit mRNA translation in infected cells or recruit a cellular protein to enhance its own replication.

Despite major advances in diagnosing chronic hepatitis C and screening the blood supply since that time, almost nothing is known about how the virus infects, kills or transforms cells. For this reason, current therapeutic options are limited and new agents have been difficult to develop.

According to a recent National Institutes of Health Consensus Development Conference Panel Statement on the Management of Hepatitis C (5), there is an urgent need for effective antiviral therapeutics capable of inhibiting HCV replication and stopping or delaying the progression of liver disease. The Panel also concluded that a major bottleneck to the drug discovery process is the absence of a readily available cell culture system that is fully permissive for viral replication. A small animal model of HCV infection is also lacking. For these reasons, novel, alternative approaches must be developed to identify targets for the design of therapeutic agents for the treatment of patients with chronic hepatitis C.

The development of specific drugs against HCV has been impeded because there is no non-primate animal model of

infection and all attempts to culture the virus have failed. Currently, the only currently approved drugs in the United States are preparations of interferon-alpha and ribavirin. The long-term cure rate of subjects treated with interferon-alpha is less than 10%. The use of ribavirin, in combination with interferon-alpha, has shown slightly better long-term cure, however, still in only a minority of subjects.

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Summary of the Invention

5 The present invention provides a method of treating or preventing hepatitis C virus infection in a subject which comprises administering an effective amount of an agent to the subject, wherein the agent is capable of specifically binding to the HCV core protein so as to inhibit hepatitis C virus replication. The present invention provides a method of identifying a compound which can inhibit the functions of hepatitis C virus, wherein said compound
10 inhibits hepatitis C virus replication by inhibiting the interactions between HCV core protein and an agent capable of specifically binding to said HCV core protein, comprising: (a) incubating said compound, the HCV core protein and said agent under a suitable reaction
15 conditions, (b) determining the binding between the HCV core protein and said agent in the presence of said compound, and (c) comparing the binding in step (b) with the binding between the HCV core protein and said agent in the absence of said compound so as to identify a compound
20 which can inhibit the replication of hepatitis C virus by inhibiting the binding between HCV core protein and said agent.

25 The present invention also provides a method for determining whether a compound can treat or prevent hepatitis C virus infection in a subject, comprising (a) incubating said compound, the HCV core protein and an agent capable of specifically binding to said HCV core protein, (b) determining the binding between the HCV core
30 protein and said agent in the presence of said compound, and (c) comparing the binding in step (b) with the binding between the HCV core protein and said agent in the absence of said compound so as to identify a compound which can treat or prevent hepatitis C virus infection in a subject,
35 wherein said compound treats or prevents hepatitis C virus

infection by specifically inhibiting the binding of HCV core protein and said agent so as to suppress hepatitis C virus replication.

- 5 The present invention further provides a composition for inhibiting cell growth, comprises a HCV core protein or a variant thereof, wherein said HCV core protein or its variant inhibit cancer cell growth by inhibiting the cellular DEAD box proteins.

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Brief Description of the Figures

- Figure 1: Diagram of the major processed proteins encoded by the HCV genome. The 3010-3033 amino acid polyprotein is processed into several smaller polypeptides. Core, E1 and E2 are structural polypeptides. Core protein is the virus nucleocapsid and E1 and E2 are viral envelope proteins. The major non-structural proteins are NS2, NS3, NS4 and NS5. NS4 is further processed into NS4A and NS4B and NS5 into NS5A and NS5B. NS2 and part of NS3 are proteases that process the viral polyprotein. NS3 also has RNA-helicase activity. NS4A is a cofactor for the NS3 protease and NS5B is an RNA-dependent, RNA polymerase. The functions of NS4B and NS5A are less-well understood but NS5A is thought to play a role in determining sensitivity to interferon.
- Figure 2: Amino acid sequence of human DBX (alternative transcript 2), SEQ ID NO:1. The domain that binds to HCV core protein in the yeast two-hybrid assay is underlined and separately listed as SEQ ID NO:2.
- Figure 3: Amino acid sequence of human DBX (alternative transcript 2) from amino acid 509 to amino 645 (SEQ ID NO:3) that binds to HCV core protein in the yeast two hybrid assay.
- Figure 4: Complete cDNA sequence of human epsilon 14-3-3 protein (SEQ ID NO:4) and amino acid sequence translation thereof (SEQ ID NO:5).
- Figure 5: Binding of DBX to HCV core protein *in vitro*.

5A: A standard amount of ^{35}S -HCV core protein (amino acids 1-123), 10% of which is shown in the autoradiogram (lane 1), was used in each binding assay. ^{35}S -HCV core protein was incubated with glutathione-Sepharose (lane 2), 20 μg of GST coupled to glutathione-Sepharose (lane 3) in binding buffer containing 0.15 M NaCl and 0.2 μg of GST-DBX fusion protein coupled to glutathione-Sepharose in buffers containing the NaCl concentrations indicated above each lane (lanes 4-8). Glutathione-Sepharose was then washed with buffer containing the indicated NaCl concentration and the bound proteins were eluted with 4% SDS, subjected to SDS-polyacrylamide gel electrophoresis and detected by autoradiography of dried slabs gels.

5B: Binding assay similar to that shown in panel A in which GST-DBX fusion protein was incubated with ^{35}S -HCV core protein in buffers containing 0.15 M NaCl and 0.05% to 1.0% of Nonidet P-40 (NP-40) (lanes 4-6). Migrations of molecular mass standards are indicated in kilodaltons at the left of each panel.

Figure 6: Primary structures of DBX, PL10 and Ded1 and their interactions with HCV core protein in the yeast two-hybrid assay.

6A: Alignment of deduced amino acid sequences of DBX (GenBank Accession number AF000982), PL10 (GenBank Accession number J04847) and Dedlp (GenBank Accession number X57278) is shown. Identical amino acids are shown as white on cyan. Conserved substitutions are shown as black on magenta. Dots represent gaps to optimize alignments which were obtained using the Pileup program.

6B: Two-hybrid assays showing interaction of HCV core protein with DBX and PL10 but not with Dedlp. Yeast strain Y190 was co-transformed with a plasmid expressing the cytoplasmic domain of HCV fused to the GAL4 DNA binding domain and plasmids expressing either a portion of DBX or the corresponding portions of PL10 or Dedlp fused to the GAL4 transcriptional activation domain. Transformants giving β -galactosidase activity (positive interactions) are blue. Control reactions of DBX, PL10 and Dedlp GAL 4 activation domain fusion proteins with GAL4 DNA binding domain alone were negative (data not shown).

Figure 7: Immunofluorescence localization of DBX and HCV core protein in mammalian cells.

7A: Hela cells were transiently transfected with

cDNA encoding FLAG-tagged HCV core protein (left panel) or with cDNA encoding myc-tagged DBX (right panel). Cells were incubated with monoclonal anti-FLAG or anti-myc (9E10) antibody followed by rhodamine-conjugated secondary antibody. HCV core protein appears primarily in large, discrete foci at the endoplasmic reticulum membrane whereas DBX has a more diffuse cytoplasmic localization.

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7B: Co-localization of DBX and HCV core protein in COS-7 cells transiently transfected to express both FLAG-tagged HCV core protein and myc-tagged DBX. All cells were fixed and incubated with the same combination of rabbit anti-FLAG polyclonal antibody and mouse anti-myc monoclonal (9E10) antibody followed by both fluorescein isothiocyanate-conjugated goat anti-rabbit and rhodamine-conjugated goat anti-mouse antibodies. Cells transfected to express FLAG-tagged HCV core protein alone (left column) showed essentially only green fluorescence resulting from anti-FLAG and fluorescein isothiocyanate-conjugated antibody labeling (row G). Cells transfected to express myc-tagged DBX alone (middle column) showed essentially only red fluorescence resulting from anti-myc and rhodamine conjugated antibody labeling (row R). The right column shows COS-7 cells co-

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transfected to express both FLAG-tagged HCV core protein (row G) and myc-tagged DBX (row R). Merged images (row M) appear yellow where green fluorescence corresponding to HCV core protein localization and red fluorescence corresponding to DBX localization overlap.

Figure 8: Inhibition of DBX and PL10 but not Ded1p by HCV core protein. Yeast strains with chromosomal *ded1* deletion complemented with either *DBX*, *PL10*, or *DED1* cDNAs driven by the yeast GPD promoter on centromeric plasmids were transformed with a plasmid that expressed full-length HCV core protein (*top*) or control plasmid p423GPD (*bottom*). The resulting transformants were spread on histidine, leucine drop-out plates and incubated at 30°C for 7 days and photographs (negatives are shown) were taken of each plate. Note colony growth of all yeast strains transfected with control plasmid (*bottom* panels). In contrast, *DBX*- and *PL10*-complemented *ded1*-deletion strains do not demonstrate significant colony growth when HCV core protein is expressed whereas growth of the *DED1*-complemented strain is unaffected (*top* panels).

Figure 9: Inhibition of translation of capped mRNA in

vitro by HCV core protein. Rabbit reticulocyte lysates were incubated with glutathione-Sepharose beads loaded with either 300 ng of a GST-HCV core fusion protein or GST. *In vitro* synthesized capped or non-capped luciferase mRNAs were translated at 30°C for 90 minutes and luciferase activity was measured. Results are expressed as the relative luciferase activities produced in reticulocyte lysates treated equal concentrations of GST-HCV core fusion protein (shaded bars) or GST (open bars, arbitrarily assigned 100% activity). Values shown are means \pm standard errors (n = 6). *p < 0.0001: no significant difference.

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Detailed Description of the Invention

The present invention provides a method of treating or preventing hepatitis C virus infection in a subject which comprises administering an effective amount of an agent to the subject, wherein the agent is capable of specifically binding to the HCV core protein so as to inhibit hepatitis C virus replication.

In one embodiment of the method, the agent is capable of specifically binding to the HCV core protein having an amino acid sequence of Figure 2, SEQ ID NO:1.

As used herein, "inhibition of hepatitis C virus replication" means interrupting or stopping the growth or multiplication of the hepatitis C virus.

In another embodiment of the method, the binding of the agent to hepatitis C virus core protein prevents the hepatitis C virus from attaching to the cells of the subject so as to inhibit hepatitis C virus replication.

In another embodiment of the method, the binding of the agent to hepatitis C virus core protein prevents the hepatitis C virus from entering into the cells of the subject so as to inhibit hepatitis C virus replication.

In another embodiment of the method, the agent binds to the cytoplasmic domain of HCV core protein which comprises amino acid residues 1-123 of said HCV core protein of Figure 2, SEQ ID NO:1.

In another embodiment of the method, the agent is a polypeptide, a pseudo enzyme, a peptidomimetic compound, a nucleic acid molecule, an antibody or variant thereof.

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In another embodiment of the method, the agent comprises a cellular protein.

5 In another embodiment of the method, the cellular protein comprises a DEAD-box protein, or a 14-3-3 protein.

In another embodiment of the method, the DEAD box protein comprises a DEAD box RNA helicase.

10 In another embodiment of the method, the DEAD-box RNA helicase is a human DEAD-box protein DBX or a variant thereof.

15 Variants in amino acid sequence of human DEAD-box protein DBX are produced when one or more amino acids in naturally occurring human DEAD-box protein DBX is substituted with a different natural amino acid, an amino acid derivative, a synthetic amino acid, an amino acid analog or a non-native amino acid. Particularly preferred variants include
20 homologous human DEAD-box protein DBX. Variants of a human DEAD-box protein DBX may include biologically active fragments of naturally occurring human DEAD-box protein DBX, wherein sequences of the variant differ from the wild type human DEAD-box protein DBX sequence by one or more
25 conservative amino acid substitutions. Such substitutions typically would have minimal influence on the secondary structure and hydrophobicity of human DEAD-box protein DBX.

30 In another embodiment of the method, the human DEAD-box protein DBX has the amino acid sequence of Figure 2, SEQ ID NO:1.

"Polypeptide" includes both peptides and proteins.
"Peptide" means a polypeptide of fewer than 10 amino acid
35 residues in length, and "protein" means a polypeptide of 10

or more amino acid residues in length. In this invention, the polypeptides may be naturally occurring or recombinant (i.e. produced via recombinant DNA technology), and may contain mutations (e.g. point, insertion and deletion mutations) as well as other covalent modifications (e.g. glycosylation and labeling via biotin, streptavidin, fluoracine, and radioisotopes such as ¹³¹I). Moreover, each instant composition may contain more than a single polypeptide, i.e. each may be a monomer (one polypeptide bound to a polymer) or a multimer (two or more polypeptides bound to a polymer or to each other).

As used herein, "effective amount" means an amount of a compound which interrupts the binding between hepatitis C virus core protein and a cellular protein, which can inhibit the hepatitis C virus replication and can be determined using methods well known to those skilled in the art.

In another embodiment of the method, the variant of human DEAD-box protein DBX comprises the amino acid sequence of Figure 2, SEQ ID NO:2.

In another embodiment of the method, the variant of human DEAD-box protein DBX comprises the amino acid sequence of Figure 3, SEQ ID NO:3.

In another embodiment of the method, the hepatitis C virus infects the liver of the subject.

In another embodiment of the method, the hepatitis C virus infects the liver of a human.

In another embodiment of the method, the variant of the human DEAD-box protein DBX comprises 100-200 amino acid

residues which mimics the amino acid sequence of Figure 2, SEQ ID NO:2 or the amino acid sequence of Figure 3, SEQ ID NO:3.

- 5 In another embodiment of the method, the 14-3-3 protein comprises the amino acid sequence of Figure 4, SEQ ID NO:5 or a variant thereof.

10 Variants in amino acid sequence of 14-3-3 protein are produced when one or more amino acids in naturally occurring 14-3-3 protein is substituted with a different natural amino acid, an amino acid derivative, a synthetic amino acid, an amino acid analog or a non-native amino acid. Particularly preferred variants include homologous
15 14-3-3 protein. Variants of a 14-3-3 protein may include biologically active fragments of naturally occurring 14-3-3 protein, wherein sequences of the variant differ from the wild type 14-3-3 protein sequence by one or more conservative amino acid substitutions. Such substitutions
20 typically would have minimal influence on the secondary structure and hydrophobicity of the 14-3-3 protein.

In another embodiment of the method, the variant of said 14-3-3 protein comprises 50-200 amino acid residues which
25 mimics the active site of said 14-3-3 protein of Figure 4, SEQ ID NO:5.

In another embodiment of the method, the agent comprises nucleic acid molecule encoding DEAD-box protein of Figure
30 4, SEQ ID NO:5 or a variant thereof.

In yet another embodiment of the method, the agent comprises nucleic acid molecule encoding 14-3-3 protein of Figure 4, SEQ ID NO:5 or a variant thereof.

In a further embodiment of the method, the agent is administered with a pharmaceutically acceptable carrier.

5 The present invention provides a method of identifying a compound which can inhibit the replication of HCV, wherein said compound inhibits hepatitis C virus replication by inhibiting the binding between HCV core protein and an agent capable of specifically binding to said HCV core protein, comprising: (a) incubating said compound, the HCV
10 core protein and said agent under a suitable reaction conditions, (b) measuring the binding between the HCV core protein and said agent in the presence of said compound, and (c) comparing the binding in step (b) with the binding
15 between the HCV core protein and said agent in the absence of said compound so as to identify a compound which can inhibit the replication of hepatitis C virus by inhibiting binding between HCV core protein and said agent.

20 In one embodiment of the method, the agent is known to bind to HCV core protein.

In another embodiment of the method, the binding of the agent to hepatitis C virus core protein prevents the hepatitis C virus from attaching to the cells of the
25 subject so as to inhibit hepatitis C virus replication.

In another embodiment of the method, the binding of the agent to hepatitis C virus core protein prevents the hepatitis C virus from entering into the cells of the
30 subject so as to inhibit hepatitis C virus replication.

In another embodiment of the method, the inhibition of HCV replication is in a subject.

35 In another embodiment of the method, the agent is a

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polypeptide, a pseudo enzyme, a peptidomimetic compound, a nucleic acid molecule, an antibody or variant thereof.

5 In another embodiment of the method, the agent comprises a cellular protein.

In another embodiment of the method, the cellular protein comprises a DEAD-box protein, or a 14-3-3 protein.

10 In another embodiment of the method, the DEAD box protein comprises a DEAD box RNA helicase.

15 In another embodiment of the method, the DEAD-box RNA helicase comprises a human DEAD-box protein DBX or a variant thereof.

20 In another embodiment of the method, the human DEAD-box protein DBX comprises the amino acid sequence of Figure 2 SEQ ID NO:1.

In another embodiment of the method, the variant of the human DEAD-box protein DBX comprises the amino acid sequence of Figure 2, SEQ ID NO:2.

25 In another embodiment of the method, the variant of the human DEAD-box protein DBX comprises the amino acid sequence of Figure 3, SEQ ID NO:3.

30 In another embodiment of the method, the variant of the human DEAD-box protein DBX comprises 100-200 amino acid residues which mimics the amino acid sequence of Figure 2, SEQ ID NO:2 or the amino acid sequence of Figure 3, SEQ ID NO:3.

35 In another embodiment of the method, the 14-3-3 protein

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In another embodiment of the method, the variant of said
5 14-3-3 protein comprises 50-200 amino acid residues which
mimics the active site of said 14-3-3 protein of Figure 4,
SEO ID NO:5.

In another embodiment of the method, the agent comprises nucleic acid molecule encoding 14-3-3 protein of Figure 4, SEQ ID NO:5 or a variant thereof.

20 In another embodiment of the method, the inhibition of hepatitis C virus replication is *in vitro*.

In another embodiment of the method, the subject is a human.

In another embodiment of the method, the inhibition of hepatitis C virus replication occurs in the liver of a human.

In another embodiment of the method, the binding between the HCV core protein and the agent is measured by yeast two-hybrid screening.

- 5 In yet another embodiment of the method, the compound is not previously known.

In a further embodiment of the method, the previously unknown compound is identified by the method.

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The present invention also provides a composition comprising an effective amount of the compound identified by the method which is capable of inhibiting the binding between hepatitis C virus core protein and a cellular

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The present invention also provides a pharmaceutical composition comprising an effective amount of the compound identified by the method which is capable of treating or preventing hepatitis C virus infection.

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The present invention also provides a method for determining whether a compound can treat or prevent hepatitis C virus infection in a subject, comprising (a) incubating said compound, the HCV core protein and an agent capable of specifically binding to said HCV core protein, (b) determining the binding between the HCV core protein and said agent in the presence of said compound, and (c) comparing the binding in step (b) with the binding between the HCV core protein and said agent in the absence of said compound so as to identify a compound which can treat or prevent hepatitis C virus infection in a subject, wherein said compound treats or prevents hepatitis C virus infection by specifically inhibiting the binding of HCV core protein and said agent so as to suppress hepatitis C

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virus replication.

In one embodiment of the method, the agent is known to bind to HCV core protein.

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In another embodiment of the method, the binding of the agent to hepatitis C virus core protein prevents the hepatitis C virus from attaching to the cells of the subject so as to treat or prevent hepatitis C virus infection.

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In another embodiment of the method, the binding of the agent to hepatitis C virus core protein prevents the hepatitis C virus from entering into the cells of the subject so as to treat or prevent hepatitis C virus infection.

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In another embodiment of the method, the subject is a mammal.

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In another embodiment of the method, the subject is a human.

In another embodiment of the method, the hepatitis C virus replication occurs in the liver of the subject.

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In another embodiment of the method, the hepatitis C virus replication occurs in the liver of a human.

In another embodiment of the method, the compound can be administered orally or by injection.

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In another embodiment of the method, the compound is not previously known.

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In another embodiment of the method, the previously unknown compound is identified by said method.

5 In another embodiment of the method, the agent is a polypeptide, a pseudo enzyme, a peptidomimetic, a nucleic acid, an antibody or variant thereof.

10 In another embodiment of the method, the agent comprises a cellular protein.

In another embodiment of the method, the cellular protein comprises a DEAD-box protein, or a 14-3-3 protein.

15 In another embodiment of the method, the DEAD box protein comprises a DEAD box RNA helicase.

20 In another embodiment of the method, the DEAD-box RNA helicase comprises a human DEAD-box protein DBX or a variant thereof.

In another embodiment of the method, the human DEAD-box protein DBX comprises the amino acid sequence of Figure 2 SEQ ID NO:1.

25 In another embodiment of the method, the variant of the human DEAD-box protein DBX comprises the amino acid sequence of Figure 2, SEQ ID NO:2.

30 In another embodiment of the method, the variant of the human DEAD-box protein DBX comprises the amino acid sequence of Figure 3, SEQ ID NO:3.

35 In another embodiment of the method, the variant of the human DEAD-box protein DBX comprises 100-200 amino acid residues which mimics the amino acid sequence of Figure 2,

SEQ ID NO:2 or the amino acid sequence of Figure 3, SEQ ID NO:3.

In another embodiment of the method, the 14-3-3 protein
5 comprises the amino acid sequence of Figure 4, SEQ ID NO:5 or a variant thereof.

In another embodiment of the method, the variant of said
14-3-3 protein comprises 50-200 amino acid residues which
10 mimics the active site of said 14-3-3 protein, Figure 4, SEQ ID NO:5.

In another embodiment of the method, the agent comprises
nucleic acid molecule encoding DEAD-box protein of Figure
15 2, SEQ ID NO:1 or a variant thereof.

In yet another embodiment of the method, the agent
comprises nucleic acid molecule encoding 14-3-3 protein of
Figure 4, SEQ ID NO:5 or a variant thereof.

20 In a further embodiment of the method, the agent comprises administered with a pharmaceutically acceptable carrier.

The present invention further provides a composition for
25 inhibiting cell growth, comprises a HCV core protein or a variant thereof, wherein said HCV core protein or its variant inhibit cancer cell growth by inhibiting the cellular DEAD box proteins.

30 In one embodiment of the composition, the growth of cancer cells are inhibited.

As used herein, "subject" means any animal, including, for
example, mice, rats, dogs, guinea pigs, ferrets, rabbits,
35 and primates. In the preferred embodiment, the subject is

a human.

"Cells" mean any cells, including, for example, lung cells, and kidney cells. In the preferred embodiment, the cells
5 are liver cells. In a more preferred embodiment, the cells are human liver cells.

"Attachment" means the state of being firmly attached or bound through chemical or physical interactions or both.
10 "Attachment of hepatitis C virus onto cells" means the hepatitis C virus being firmly attached or bound to the cell surface through the interaction between hepatitis C virus proteins, such as HCV core protein, and the HCV receptors located at the surface of the cells.

15 "Entry of HCV into cells" means the penetration of hepatitis C virus through the cell membrane into the cells from cell surface.

20 "Assembly" means association, binding, packing or aggregation. "HCV assembly" means the association of the single stranded HCV RNA with HCV coat proteins, the formation of the single stranded HCV RNA - Coat Protein complex, or the packing of coat proteins onto the single
25 stranded HCV RNA.

"HCV replication" means HCV reproduction within the cells.

30 "Hepatitis C virus infection" comprises the attachment of hepatitis C virus to cell surface, the entry of hepatitis C virus into cells, the replication of hepatitis C virus within the cells, and the death or transformation of the cells.

35 "Agent" means any biological molecule which specifically

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binds to HCV core protein. In one embodiment, the agent comprises a cellular protein.

5 "Nucleic acid molecule" means any natural or synthetic cDNA or mRNA.

As used herein, "suitable reaction conditions" means conditions under which an agent competitively bind to HCV core protein or a variant thereof.

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This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

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Experimental Details

Yeast Two-Hybrid Screening: The Matchmaker Two-Hybrid System 2 was used to screen human liver Matchmaker cDNA library HL4002AB (Clontech) with the cytoplasmic domain (amino acids 1 to 123 that precedes the first predicted transmembrane segment) of HCV core protein as bait in the yeast two-hybrid assay (19). Library screening was performed using previously described methods (20,29). To construct the bait plasmid, DNA encoding amino acids 1-123 of HCV core protein (numbering as in reference 8) was amplified by PCR with pHCV-1 (12), provided by M. Houghton (Chiron Corporation), as template. The HCV sequences in pHCV-1 derive from a library made from the plasma of an infectious chimpanzee (12). The amplified DNA was cloned into the GAL4 DNA binding domain fusion vector pAS2-1 (Clontech) to yield pAS2-1-HCV-core₁₋₁₂₃. *Saccharomyces cerevisiae* strain Y190 was sequentially transformed with pAS2-1-HCV-core₁₋₁₂₃ and library recombinants in the GAL4 activation domain fusion vector pACT2 (Clontech). Positive pACT2-derived plasmids were rescued and used to co-transform yeast with pAS2-1-HCV-core₁₋₁₂₃, pLAM5'-1 (Clontech) and pAS2-1 to confirm the specificity of the reactions. For analysis of PL10 and Ded1p binding, cDNAs encoding PL10 from amino acids 408 to 660 and Ded1p amino acids 368 to 604 (corresponding to the longest portion of DBX isolated in the two-hybrid screen) were amplified by PCR from template plasmids (23). The amplified cDNAs were cloned into pACT2 and used to co-transform yeast with pAS2-1-HCV-core₁₋₁₂₃. DNA sequencing of isolated library plasmid inserts and the bait constructs was performed on a 373A Sequencer (Applied Biosystems) at the Columbia University Cancer Center DNA core facility. Sequence analysis was performed using the Wisconsin Package (Genetics Computer Group) and applications available via the Internet at the National Center for Biotechnology Information World Wide

Web site (<http://www.ncbi.nlm.nih.gov/>).

In Vitro Binding Assays: A PCR product encoding the cytoplasmic domain of HCV core protein (amino acids 1-123) was cloned into pBFT4 for *in vitro* transcription-translation (26). DBX cDNA encoding amino acids 409 to 662 was excised from plasmid pACT2 by restriction endonuclease digestion and cloned into pGEX2T (Pharmacia Biotech) to yield pGEX2T-DBX409-662 that expressed a glutathione-S-transferase (GST) fusion protein in *Escherichia coli*. Plasmid construction was confirmed by DNA sequencing. *In vitro* transcription-translation was performed with the TNT T7 Coupled Reticulocyte Lysate System (Promega) using L-[³⁵S]-methionine (NEN). Binding assays were performed as described previously (26).

Cell Transfection and Confocal Immunofluorescence

Microscopy: A PCR product encoding full-length HCV core protein (amino acids 1-191) obtained using pHCV-1 (12) as template was cloned in-frame into pBFT4 which contains an initiation codon and FLAG tag 5' to the cloning site. A DNA fragment was isolated by restriction endonuclease digestion at sites flanking the initiation and termination codons and cloned into pSVK3 (Pharmacia Biotech) to obtain pSVK3-FLAG-core for expression of HCV core protein with a FLAG tag at its amino-terminus. To obtain full-length DBX cDNA, PCR was performed using a Marathon-ready cDNA human liver library (Clontech) as template to amplify the first 1439 nucleotides of DBX cDNA which was ligated in-frame into pGEX2T-DBX409-662 to produce pGEX2T-DBX. The coding region of pGEX2T-DBX was isolated by restriction endonuclease digestion and cloned into pBluescript II SK⁻ (Stratagene) to produce pBluescript-DBX. A cDNA containing the 3' 668 nucleotides of DBX, excluding the stop codon,

was amplified by PCR and ligated into pBluescript II-DBX to replace the corresponding nucleotides. The entire DBX coding region was then excised by restriction endonuclease digestion and ligated into pcDNA3.1(-)/Myc-His A (Invitrogen) to produce pcDNA3.1/His A-DBX-myc that encoded full-length DBX with a c-myc tag at its carboxyl-terminus. All plasmid constructs were confirmed by DNA sequencing. HeLa or COS-7 cells (ATCC) grown on glass slides were transfected with pSVK3-FLAG-core, pcDNA3.1/HIS A-DBX-myc or both using Tfx-20 (Promega) or DMRIE-C (Life Technologies). Cells were washed in phosphate-buffered saline 48 hours after transfection and fixed with methanol for 5 min at -20°C followed by acetone at -20°C for twenty seconds. Indirect immunofluorescence microscopy was performed as described (30). To detect express FLAG-tagged proteins in double-labeling experiments, FLAG-probe (Santa Cruz Biotechnology), a rabbit polyclonal antibody, was used. To reduce background, FLAG-probe was incubated with COS-7 cells fixed with methanol/acetone at a 1:100 dilution for twelve to sixteen hours prior to use. Anti-FLAG M2 monoclonal antibody (Eastman Kodak) was used in single-labeling experiments at a 1:200 dilution. Monoclonal anti-c-myc antibody 9E10 (Babco) was used at a 1:1000 dilution. Fluorescein isothiocyanate-conjugated goat anti-rabbit IgG and rhodamine-conjugated goat anti-mouse IgG secondary antibodies were obtained from Jackson Immuno Research Laboratories. Microscopy was performed using a Zeiss LSM 410 confocal laser scanning system attached to Zeiss Axiovert 100TV inverted microscope (Carl Zeiss). Images were processed using Photoshop software (Adobe) on a Macintosh G3 computer (Apple Computer).

Yeast Strains: Yeast strain YTC83 [MATa *ded1::TRP1 ura3-52 lys2-801 ade2-101 trp1-D1 his3-D200 leu2-D1 pPL1004 (PL10/CEN/LEU2)*], which contains a chromosomal *ded1*

deletion complemented by *PL10* cDNA (23). To obtain a yeast strain with a chromosomal *ded1* deletion complemented by *DBX* cDNA, full-length *DBX* cDNA was excised from pGEX2T-*DBX* by restriction endonuclease digestion and ligated into pRS315pG1. This plasmid was used to transform yeast strain YTC75 [MATa *ded1::TRP1 ura3-52 lys2-801 ade2-101 trp1-D1 his3-D200 leu2-D1* pDED1008 (*DED1/CEN/URA3*)] (23), which was then grown on leucine dropout plates. Transformants were replica-plated onto 5-fluoroorotic acid plates as described (31) to yield strain YNM1DX. To obtain a yeast strain with a chromosomal *ded1* deletion complemented by *DED1* cDNA driven by a glyceraldehyde-3-phosphate (GPD) promoter on a centromeric plasmid, the native promoter, 5' untranslated region and part of the 5' coding region were excised by restriction endonuclease digestion from pDED1009 (*DED1/CEN/LEU2*). The GPD promoter, isolated from pRS315pG1 by restriction endonuclease digestion, and 477 5' coding nucleotides of *DED1*, amplified by PCR, were then sequentially ligated into this pDED1009-derived plasmid to yield pDED_{GPD}. Yeast strain YTC75 was then transformed with pDED_{GPD} and 5-fluoroorotic acid counter-selection performed to obtain strain YNM1DD [MAT a *ded1::TRP1 ura3-52 lys2-801 ade2-101 trp1-D1 his3-D200 leu2-D1* pDED_{GPD} (*DED1/CEN/LEU2*)]. All constructs were confirmed by DNA sequencing.

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Effects of HCV Core Protein on Growth of Yeast Strains: The coding region for full-length HCV core protein (amino acid 1-191) was excised from pBFT4 by restriction endonuclease digestion and ligated into p423GPD (ATCC) to produce p423GPD-core. The coding region for the cytoplasmic domain of HCV core protein (amino acid 1-123) was also ligated into p423GPD to yield p423GPD-core₁₋₁₂₃. Constructs were confirmed by DNA sequencing. Yeast strains YTC83, YNM1DX and YNM1DD were transformed with p423GPD, p423GPD-core and

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p423GPD-core₁₋₁₂₃ using the lithium acetate-mediated method (24) and grown on histidine-leucine dropout plates for seven days. Plates were photographed to record colony growth.

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Effect of HCV Core Protein on In Vitro Translation: cDNA encoding HCV core protein from amino acids 1 to 123 was ligated into pGEX4T-3 (Pharmacia) to produce a GST fusion protein (GST-core₁₋₁₂₃) in *Escherichia coli*. Plasmid construction was confirmed by DNA sequencing. pGEM-luc (Promega) was linearized with XhoI and used as a template for luciferase RNA transcription with the RiboMAX RNA Production System-SP6 (Promega). When capped RNA was synthesized, 3 mM of ⁷mGpppG (New England Biolabs) was included in the reaction mixture. The DNA template was removed by digestion with DNase following the transcription reaction and synthesized mRNA was purified using the RNeasy Mini Kit (Qiagen). For *in vitro* translation, 16.5 µl of Flexi Rabbit Reticulocyte Lysate (Promega) was used and incubated for one hour at 4°C with 8.25 µl of glutathione-Sepharose 4B (Pharmacia Biotech) loaded with either 300 ng of GST-core₁₋₁₂₃ or GST followed by centrifugation for 5 minutes at 2000 x g. Translation reactions were then performed according to the manufacturer's instructions and luciferase activity measured by luminescence emission using the Luciferase Assay System (Promega).

HCV Core Protein Binding to DBX: Screening of 8 x 10⁶ recombinants of a human liver cell cDNA library with the cytoplasmic domain of HCV core protein as bait in the yeast two-hybrid assay led to the isolation of 5 positive clones, 3 of which encoded portions of DBX, the longest from amino acid 409 to amino acid 662. The two other positive clones encoded portions of epsilon 14-3-3 (See Figure 4), a member of the 14-3-3 family of proteins that have numerous

proposed functions, including activities in signal transduction. DBX is the human orthologue of the mouse DEAD-box protein PL10 (21-22, 32). PL10 is the functional orthologue of *Saccharomyces cerevisiae* Ded1p, an ATP-dependent RNA helicase for capped mRNA (23). DBX is 95% identical in primary structure to PL10 and 54% identical to Ded1p (Fig. 6A). In the yeast two-hybrid assay, HCV core protein interacts with DBX and PL10 but not with Ded1p (Fig. 6B).

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We confirmed the interaction between HCV core protein and DBX in an *in vitro* binding assay. The cytoplasmic domain of HCV core protein was synthesized by *in vitro* translation and incubated with GST or a GST-fusion protein containing DBX from amino acid 409 to amino acid 662. Proteins were precipitated with glutathione-Sepharose and HCV core protein binding was analyzed by autoradiography. HCV core protein did not bind to GST but did bind to GST-DBX fusion protein in buffers containing NaCl concentrations as high as 1 Molar (Fig. 5A). Binding also occurred in buffers containing 1% of the non-ionic detergent Nonidet P-40 (Fig. 5B).

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Co-localization of HCV Core Protein and DBX in Cells: An interaction between HCV core protein and DBX in mammalian cells was further supported by their intracellular co-localization. Indirect confocal immunofluorescence microscopy of transfected Hela cells showed that full-length HCV core protein, which contains the cytoplasmic domain and a single transmembrane segment, was localized to the endoplasmic reticulum in discrete foci (Fig. 7A). A similar localization in the endoplasmic reticulum has been reported by others (12). Focal aggregates of HCV core protein likely arise because this polypeptide multimerizes (13). In cells not expressing HCV core protein, DBX had a

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more diffuse cytoplasmic distribution (Fig. 7A). In cells expressing HCV core protein, however, DBX was found in most instances in discrete foci that co-localized with HCV core protein (Fig. 7B). The antibodies used to detect the respective epitope tags of each protein did not cross-react significantly (Fig. 7B). HCV core protein therefore forms aggregates at the endoplasmic reticulum membrane with which DBX apparently associates.

DBX Rescues Ded1-deletion Yeast Mutants and Rescue is Prevented by HCV Core: DBX likely functions as an ATP-dependent RNA helicase for cellular mRNA, which can be inferred from its sequence similarity to mouse PL10 and yeast Ded1p (22-23). To examine the effect of HCV core protein on DBX function, we took advantage of yeast genetics and the fact that *Saccharomyces cerevisiae* has only one essential DBX-like protein, Ded1p (23). When driven by a yeast GPD promoter and carried on a centromere plasmid, mouse PL10 cDNA, as previously described (23), and DBX cDNA rescued the lethality of cells with a chromosomal *ded1* deletion. This indicates that DBX can likely function as a RNA helicase as it can replace the function of the yeast DEAD-box RNA helicase Ded1p. Expression of full-length HCV core protein severely inhibited the growth of DBX- and PL10- complemented *ded1*-deletion yeast but not *ded1*-deletion yeast complemented with *DED1* cDNA driven by the same promoter on a centromeric plasmid (Fig. 8). This is consistent with the observation that DBX and PL10, but not Ded1p, bind to HCV core protein. The cytoplasmic domain of HCV core protein that binds to DBX, without a transmembrane segment, did not significantly inhibit the growth of DBX- and PL10- complemented *ded1*-deletion yeast (data not shown), suggesting that inhibition of function may result from trapping of these proteins in aggregates at

the endoplasmic reticulum membrane (see Fig. 7).

Inhibition of In Vitro Translation of Capped mRNA by HCV

Core Protein: We examined the effects of HCV core protein
 5 on the translation of capped and uncapped luciferase RNA in
 an *in vitro* reticulocyte lysate assay. If HCV inhibits
 DBX's function as a RNA helicase, it should theoretically
 decrease the translation of capped RNA while but not
 significantly affect the translation of uncapped RNA. In
 10 the *in vitro* translation assay, the cytoplasmic portion of
 HCV core protein significantly inhibited the *in vitro*
 translation of luciferase from capped but not uncapped RNA
 (Fig. 9). Capped RNA translation was approximately four-
 fold higher than uncapped RNA translation in this assay
 15 (data not shown). This finding suggests that HCV core
 protein may inhibit the translation of capped mRNA in
 cells, presumably by inhibiting DBX function.

HCV core protein binds to the human DEAD-box protein DBX:

20 DBX rescues the lethal phenotype of *ded1*-deletion
 demonstrating that it can function as a RNA helicase for
 capped mRNA, replacing the essential yeast DEAD-box RNA
 helicase Ded1p. Our findings that HCV core protein
 prevents DBX from rescuing *ded1*-deletion yeast and that it
 25 inhibits the translation of capped RNA *in vitro* strongly
 suggest that it may inhibit cellular mRNA translation *in*
vivo. These results, however, cannot establish if
 translation inhibition occurs as a result of HCV core
 protein inhibiting DBX RNA helicase activity per se or by
 30 an interaction that results in trapping DBX at a location
 near the membrane of the endoplasmic reticulum where it
 cannot function properly. Inhibition of host cell mRNA
 translation could theoretically provide viral RNA molecules
 with enhanced access to ribosomes and the rest of the

cell's protein synthesis machinery, a phenomenon shared by several different viruses (33). A recent report has shown that high levels of expression of HCV structural and non-structural proteins is toxic to mammalian cells (34),
5 however, it is not clear if this toxicity results from inhibition of host cell translation. Because the development of a robust cell culture system to study HCV has remained elusive, it would be extremely difficult to directly investigate the effects of HCV infection on host
10 cell mRNA translation. Despite these methodological constraints limiting the ability to directly test the hypothesis, our discovery that HCV core binds to DBX and inhibits capped RNA translation in experimental assays suggests that it can similarly inhibit mRNA translation in
15 infected human cells.

DEAD-box RNA helicases unwind capped mRNA (23) and inhibition of their function should decrease translation of cellular mRNA. Inhibition of DBX function by HCV core
20 protein may only partially inhibit host mRNA translation in mammalian cells because they contain other putative RNA helicases (35). In contrast, the translation of HCV RNA, which is not capped, utilizes internal ribosome entry sites (10,11) and can be unwound by its own RNA helicase that is
25 part of the HCV NS3 protein (36,37), may proceed without DBX. This hypothetical mechanism is reminiscent of that used by poliovirus which inhibits translation factor eIF-4F (38,39) and also has RNA with internal ribosome entry sites (40). In cells, eIF-4F exists as a complex with eIF-4B,
30 which has RNA binding activity, and eIF-4A, which is also a DEAD-box RNA helicase (41). HCV and poliovirus infection may both therefore cause a decrease in the unwinding of capped mRNA in host cells.

35 In addition to inhibiting capped mRNA translation in

infected host cells, the interaction between HCV core protein and DBX may play other possible roles, including the recruitment of DBX to participate in HCV replication itself. Recruitment of host cells proteins into virions to enhance viral replication has been demonstrated in other systems. For example, the principal structural protein of the human immunodeficiency virus HIV-1 binds to cyclophilins and recruits cyclophilin A into viral particles, which appears to be necessary for efficient viral replication (42,43). In a similar fashion, recruitment of DBX into HCV particles by binding to core protein may enhance viral replication. This could theoretically occur by DBX altering viral genomic RNA structure in viral particles of in newly infected cells. Testing of this hypothesis is limited at the present time because of the lack of an efficient cell culture system for HCV.

HCV core protein has also been shown to bind to lymphotoxin- β receptor and other tumor necrosis factor receptor family members (15, 27) as well as ribonucleoprotein K (28). In our yeast two-hybrid screen, we did not isolate clones for these proteins, possibly because of subtle differences in our bait construct and the different cDNA library we used. The demonstration that other proteins interact with HCV core protein suggests that its expression in cells may have myriad consequences. Other groups (18, 44) have also reported that HCV core protein represses transcription from the p53 promoter and other eukaryotic promoters. The overall effect of HCV core protein on cell physiology under natural conditions of infection is, however, difficult to assess at the present time because of lack of a cell culture system for HCV.

Finally, it should be noted that the best current treatment

regimens for chronic hepatitis C are effective in only a minority of patients (45). If interactions between HCV and host cell proteins alter cell survival or enhance viral replication, they could be rational targets for antiviral drug design. Regardless of the physiological significance, the tight binding of any polypeptide to a structural or non-structural protein of HCV may potentially interfere with viral replication. The identification of polypeptides such as DBX that bind to HCV proteins therefore has implications for the design of compounds which may be therapeutically useful in the treatment of patients with chronic hepatitis C.

Compounds that specifically target HCV can be developed to treat HCV infection. Possible targets are the HCV proteases, RNA helicase and RNA polymerase. As the three-dimensional structures of the NS3 protease and helicase are known, rational drug design in combination with combinatorial chemistry may lead to the development of inhibitors. Other possible targets are the 5' untranslated region of the viral RNA that uses a different mechanism of ribosome entry than most host cell RNAs. The major viral structural protein (core protein, envelope proteins E1 and E2) can also be targeted for treating HCV infection. Inhibitors of core protein and envelope protein binding to critical cellular proteins could also be useful therapeutic agents.

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What is claimed is:

1. A method of treating or preventing hepatitis C virus infection in a subject which comprises administering an effective amount of an agent to the subject, wherein the agent is capable of specifically binding to the HCV core protein so as to inhibit hepatitis C virus replication.
2. The method of claim 1, wherein the hepatitis C virus infects the liver of the subject.
3. The method of claim 1, wherein the hepatitis C virus infects the liver of a human.
4. The method of claim 1, wherein the agent is capable of specifically binding to the HCV core protein having an amino acid sequence of Figure 2, SEQ ID NO:1.
5. The method of claim 1, wherein the agent binds to the cytoplasmic domain of HCV core protein which comprises amino acid residues 1-123 of said HCV core protein of Figure 2, SEQ ID NO:1.
6. The method of claim 1, wherein the agent is a polypeptide, a pseudo enzyme, a peptidomimetic compound, a nucleic acid molecule, an antibody or variant thereof.
7. The method of claim 1, wherein the agent comprises a cellular protein.
8. The method of claim 7, wherein the cellular protein comprises a DEAD-box protein, or a 14-3-3 protein.

9. The method of claim 8, wherein the DEAD box protein comprises a DEAD box RNA helicase.
- 5 10. The method of claim 9, wherein the DEAD-box RNA helicase comprises a human DEAD-box protein DBX or a variant thereof.
- 10 11. The method of claim 10, wherein the human DEAD-box protein DBX comprises the amino acid sequence of Figure 2, SEQ ID NO:1.
- 15 12. The method of claim 10, wherein the variant of the human DEAD-box protein DBX comprises the amino acid sequence of Figure 2, SEQ ID NO:2.
- 20 13. The method of claim 10, wherein the variant of the human DEAD-box protein DBX comprises the amino acid sequence of Figure 3, SEQ ID NO:3.
- 25 14. The method of claim 10, wherein the variant of the human DEAD-box protein DBX comprises 100-200 amino acid residues which mimics the amino acid sequence of Figure 2, SEQ ID NO:1 or the amino acid sequence of Figure 3, SEQ ID NO:3.
- 30 15. The method of claim 8, wherein the 14-3-3 protein comprises the amino acid sequence of Figure 4, SEQ ID NO:5 or a variant thereof.
- 35 16. The method of claim 15, wherein the variant of said 14-3-3 protein comprises 50-200 amino acid residues which mimics the active site of said 14-3-3 protein of Figure 4, SEQ ID NO:5.
17. The method of claim 1, wherein the agent comprises

nucleic acid molecule encoding DEAD-box protein of Figure 2, SEQ ID NO:1 or a variant thereof.

- 5 18. The method of claim 1, wherein the agent comprises nucleic acid molecule encoding 14-3-3 protein of Figure 4, SEQ ID NO:5 or a variant thereof.
- 10 19. The method of claim 1, wherein the agent is administered with a pharmaceutically acceptable carrier.
- 15 20. A method of identifying a compound which can inhibit the replication of HCV, wherein said compound inhibits hepatitis C virus replication by inhibiting the interactions between HCV core protein and an agent capable of specifically binding to said HCV core protein, comprising:
- 20 a) incubating said compound, the HCV core protein and said agent under a suitable reaction conditions,
- 25 b) determining the interaction between the HCV core protein and said agent in the presence of said compound, and
- 30 c) comparing the interaction in step (b) with the interaction between the HCV core protein and said agent in the absence of said compound so as to identify a compound which can inhibit the replication of hepatitis C virus by inhibiting interactions between HCV core protein and said agent.
- 35 21. The method of claim 20, wherein the agent is known to bind to HCV core protein.

22. The method of claim 20, wherein the inhibition of hepatitis C virus replication is in a subject.
- 5 23. The method of claim 22, wherein the inhibition of hepatitis C virus infection is in the liver of the subject.
- 10 24. The method of claim 22, wherein the inhibition of hepatitis C virus infection is in the liver of a human.
- 15 25. The method of claim 20, wherein the agent is a polypeptide, a pseudo enzyme, a peptidomimetic compound, a nucleic acid molecule, an antibody or variant thereof.
- 20 26. The method of claim 20, wherein the agent comprises a cellular protein.
27. The method of claim 26, wherein the cellular protein comprises a DEAD-box protein, or a 14-3-3 protein.
- 25 28. The method of claim 27, wherein the DEAD box protein comprises a DEAD box RNA helicase.
- 30 29. The method of claim 28, wherein the DEAD-box RNA helicase comprises a human DEAD-box protein DBX or a variant thereof
- 35 30. The method of claim 29, wherein the DEAD-box protein DBX comprises the amino acid sequence of Figure 2, SEQ ID NO:1.
31. The method of claim 29, wherein the variant of the

human DEAD-box protein DBX comprises the amino acid sequence of Figure 2, SEQ ID NO:2.

5 32. The method of claim 29, wherein the variant of the human DEAD-box protein DBX comprises the amino acid sequence of Figure 3, SEQ ID NO:3.

10 33. The method of claim 29, wherein the variant of the human DEAD-box protein DBX comprises 100-200 amino acid residues which mimics the amino acid sequence of Figure 2, SEQ ID NO:2 or the amino acid sequence of Figure 3, SEQ ID NO:3.

15 34. The method of claim 27, wherein the 14-3-3 protein comprises the amino acid sequence of Figure 4, SEQ ID NO:5 or a variant thereof.

20 35. The method of claim 29, wherein the variant of said 14-3-3 protein comprises 50-200 amino acid residues which mimics the active site of said 14-3-3 protein of Figure 4, SEQ ID NO:5.

25 36. The method of claim 20, wherein the agent comprises nucleic acid molecule encoding DEAD-box protein of Figure 2, SEQ ID NO:1 or a variant thereof.

30 37. The method of claim 20, wherein the agent comprises nucleic acid molecule encoding 14-3-3 protein of Figure 4, SEQ ID NO:5 or a variant thereof.

38. The method of claim 20, wherein the agent is administered with a pharmaceutically acceptable carrier.

35 39. The method of claim 20, wherein the inhibition of

hepatitis C virus replication is *in vitro*.

- 5
40. The method of claim 22, wherein the subject is a mammal.
41. The method of claim 22, wherein the subject is a human.
- 10
42. The method of claim 20, wherein the binding between the HCV core protein and the agent is measured by yeast two-hybrid screening.
- 15
43. The method of claim 20, wherein the compound is not previously known.
44. The compound identified by the method of claim 43.
- 20
45. A method for determining whether a compound can treat or prevent hepatitis C virus infection in a subject, comprising:
- 25
- a) incubating said compound, the HCV core protein and an agent capable of specifically binding to said HCV core protein under a suitable reaction conditions,
- 30
- b) determining the binding between the HCV core protein and said agent in the presence of said compound, and
- 35
- c) comparing the binding in step (b) with the binding between the HCV core protein and said agent in the absence of said compound so as to identify a compound which can treat or prevent hepatitis C virus infection in a subject, wherein said compound treats or prevents

hepatitis C virus infection by specifically inhibiting the binding of HCV core protein and said agent so as to suppress hepatitis C virus replication.

5

46. The method of claim 45, wherein the agent is known to bind to HCV core protein.

10

47. The method of claim 45, wherein the subject is a mammal.

48. The method of claim 45, wherein the subject is a human.

15

49. The method of claim 45, wherein the hepatitis C virus infection is in the liver of a subject.

50. The method of claim 45, wherein the hepatitis C virus infection is in the liver of a human.

20

51. The method of claim 45, wherein the compound can be administered orally or by injection.

25

52. The method of claim 45, wherein the compound is not previously known.

53. The method of claim 52, wherein the compound is identified by the method.

30

54. The method of claim 45, wherein the agent is a polypeptide, a pseudo enzyme, a peptidomimetic, a nucleic acid molecule, an antibody or variant thereof.

35

55. The method of claim 45, wherein the agent comprises a cellular protein.

56. The method of claim 55, wherein the cellular protein comprises a DEAD-box protein, or a 14-3-3 protein.
- 5 57. The method of claim 56, wherein the DEAD box protein comprises a DEAD box RNA helicase.
58. The method of claim 57, wherein the DEAD-box RNA helicase comprises a human DEAD-box protein DBX or a variant thereof.
- 10
59. The method of claim 58, wherein the human DEAD-box protein DBX comprises the amino acid sequence of Figure 2, SEQ ID NO:1.
- 15
60. The method of claim 58, wherein the variant of the human DEAD-box protein DBX comprises the amino acid sequence of Figure 2, SEQ ID NO:2.
- 20
61. The method of claim 58, wherein the variant of the human DEAD-box protein DBX comprises the amino acid sequence of Figure 3, SEQ ID NO:3.
- 25
62. The method of claim 58, wherein the variant of the human DEAD-box protein DBX comprises 100-200 amino acid residues which mimics the amino acid sequence of Figure 2, SEQ ID NO:2 or the amino acid sequence of Figure 3, SEQ ID NO:3.
- 30
63. The method of claim 56, wherein the 14-3-3 protein comprises the amino acid sequence of Figure 4, SEQ ID NO:5 or a variant thereof.
- 35
64. The method of claim 63, wherein the variant of said 14-3-3 protein comprises 50-200 amino acid residues

which mimics the active site of said 14-3-3 protein of Figure 4, SEQ ID NO:5.

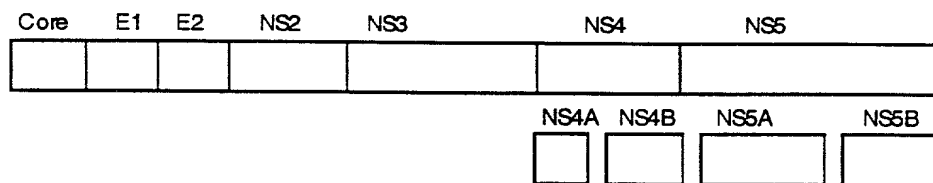
- 5 65. The method of claim 45, wherein the agent comprises nucleic acid molecule encoding DEAD-box protein of Figure 2, SEQ ID NO:1 or a variant thereof.
- 10 66. The method of claim 45, wherein the agent comprises nucleic acid molecule encoding 14-3-3 protein of Figure 4, SEQ ID NO:5 or a variant thereof.
- 15 67. The method of claim 45, wherein the agent is administered with a pharmaceutically acceptable carrier.
- 20 68. A composition comprising an effective amount of the compound identified by the method of claim 45 wherein the compound is capable of inhibiting the binding between hepatitis C virus core protein and a cellular protein.
- 25 69. A pharmaceutical composition comprising an effective amount of the compound identified by the method of claim 45, wherein the compound is capable of treating or preventing hepatitis C virus infection.
- 30 70. A composition for inhibiting cell growth, comprises a HCV core protein or a variant thereof, wherein said HCV core protein or its variant inhibit cancer cell growth by inhibiting the cellular DEAD box proteins.
- 35 71. The composition of claim 70, wherein the cell comprises cancer cell.

**HCV CORE PROTEIN BINDING AGENTS FOR
TREATMENT OF HEPATITIS C VIRUS INFECTION**

Abstract of the Disclosure

5 The present invention provides a method of treating or preventing hepatitis C virus infection in a subject which comprises administering an effective amount of an agent to the subject, wherein the agent is capable of specifically binding to the HCV core protein so as to inhibit hepatitis
10 C virus replication. The present invention also provides a method for determining whether a compound can treat or prevent hepatitis C virus infection in a subject, wherein the compound treats or prevents hepatitis C virus infection by specifically inhibiting the binding of HCV core protein
15 and said agent so as to suppress hepatitis C virus replication. The present invention further provides a composition for inhibiting cell growth, comprises a HCV core protein or a variant thereof, wherein said HCV core protein or its variant inhibit cancer cell growth by
20 inhibiting the cellular DEAD box proteins.

FIGURE 1



666260" 2E420460

FIGURE 2

SEQ. ID. NO:1

MSHVAVENALGLDQQFAGLDLNSSDNQSGGSTASKGRYIPPHLRNREATRGFYDKDSSGWSSSKDKDAYSSFGSRSDSRGKSSFFS
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RYGRRKQYPI SLVLAPTRELAVQIYEEARKFSYRSRVRPCVVYGGADIGQQIRD LERGC HLLVATPGRLVDMMERGKIGLDFCKYL
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LLDLLNATGKDSLTLVFVETKKGADSLED FLYHEGYACTSIHGDRSQRDREELH QFRSGKSPI LVATAVAARGLDISNVKHVIN F
DLPSDIEEYVHRIGRTGRVGNLGLATSF FNERNINITKD LLDLLVEAKOEVP SWLENMAYEH HYKGSSRGRSKSSRFSGGFGARDY
ROSSGASSSSFSSSRASSRS GGGGHGSSRGFGGGGYGGFYNSDGYGGNYNSQGV DWWGN

SEQ. ID. NO:2

GSTSENITQKV VVEESDKRSFLDLLNATGKDSLTLVFVETKKGADSLED FLYHEGYACTSIHGDRSQRDREELH QFRSGKSPI
LVATAVAARGLDISNVKHVINFDLPSDIEEYVHRIGRTGRVGNLGLATSF FNERNINITKD LLDLLVEAKOEVP SWLENMAYEH HY
KGSSRGRSKSSRFSGGFGARDYRQSSGASSSSFSSSRASSRS GGGGHGSSRGFGGGGYGGFYNSDGYGGNYNSQGV DWWGN

FIGURE 3

SEQ. ID. NO:3

VGSTSENITQKVWVEESDKRSFLDLLNATGKDSLTLVFVETKKGADSLEDFLYHEGYACTSIHGDRSQRDREEALHQFRSGKSP
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66550" 2420460

FIGURE 4

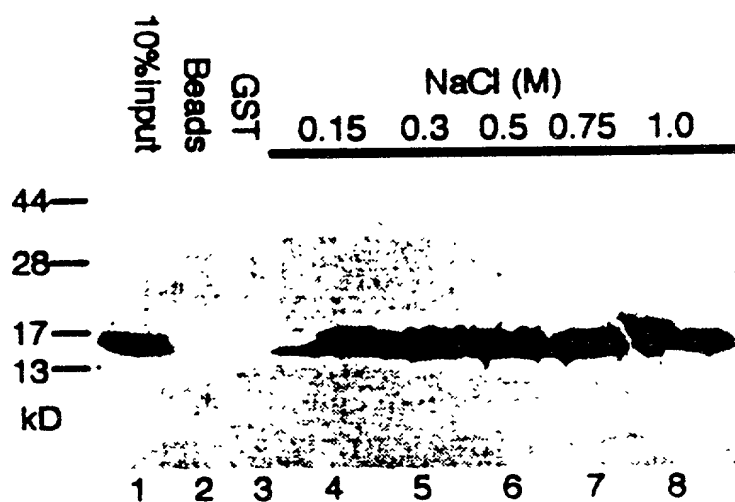
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SEQ. ID. NO:5

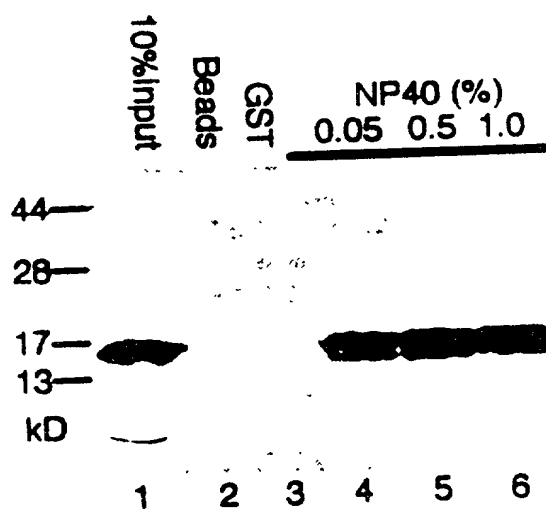
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FIGURE 5A



09407432.092999

FIGURE 5B



HCV Core Protein-DBX Interaction

1575.

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FIGURE 6B

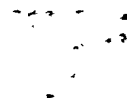
DBX



PL10

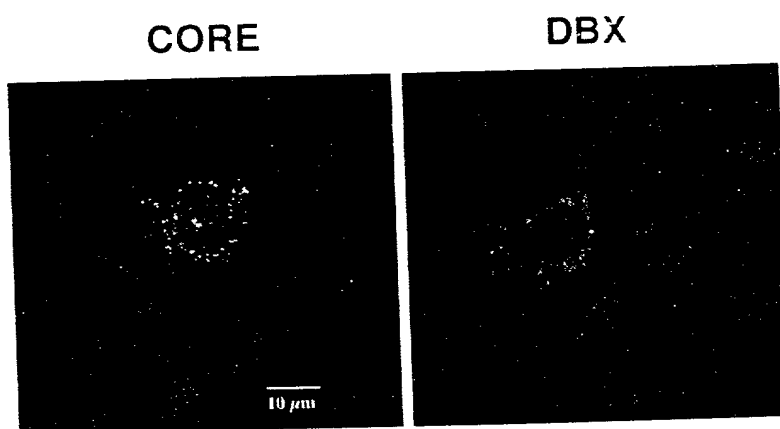


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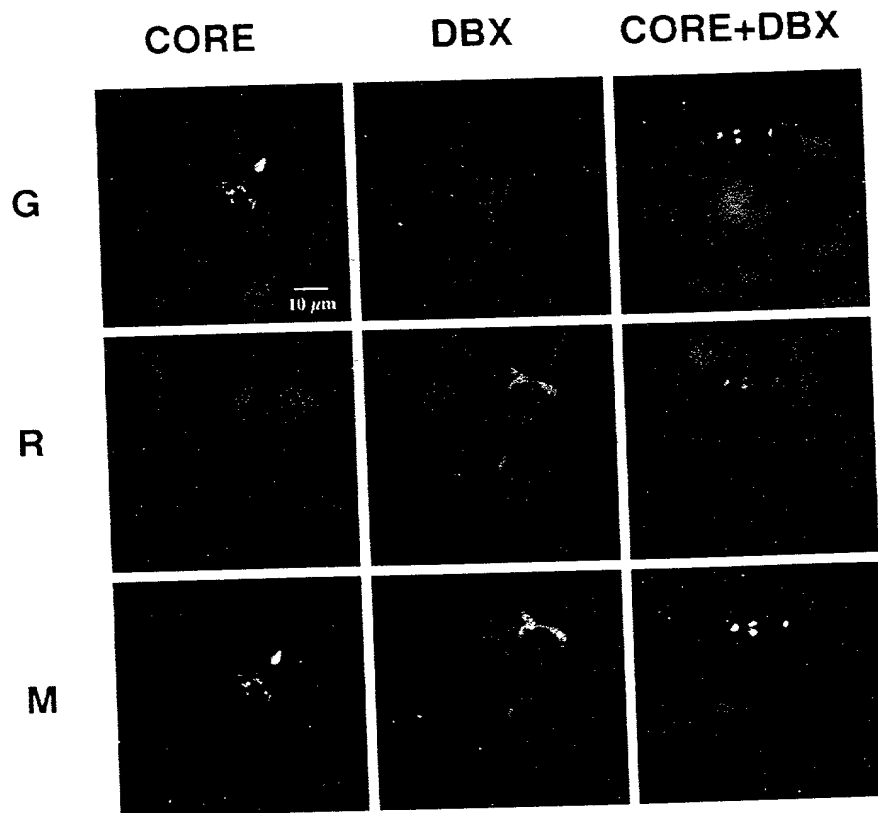
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666260" 224/20460

FIGURE 7A



665260" 224/0460

FIGURE 7B



00447430 20140460

FIGURE 8

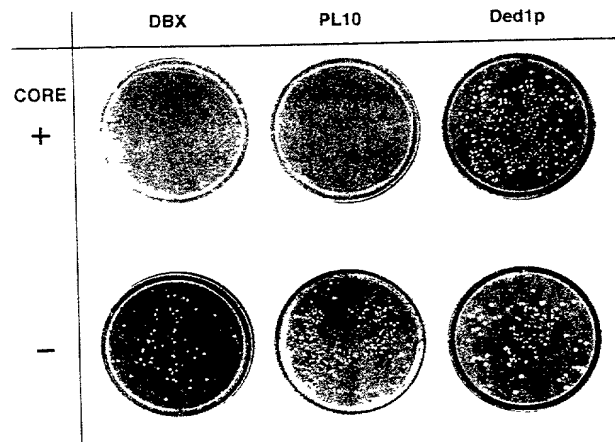


Figure 9

